Continued depression of maximal oxygen consumption and mitochondrial proteomic expression despite successful coronary artery bypass grafting in a swine model of hibernation

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Objective: Clinical studies indicate incomplete functional recovery of hibernating myocardium after coronary artery bypass grafting. We hypothesized that persistent contractile abnormalities after coronary artery bypass grafting are associated with decreased mitochondrial proteins involving electron transport chain that might limit maximal oxygen consumption.

Methods: Seven pigs with hibernating myocardium underwent off-pump revascularization with left internal thoracic artery to mid left anterior descending artery. At 4 weeks, left internal thoracic artery anastomosis was patent by multidetector computed tomography. Regional function (transthoracic echocardiography) and blood flow (microspheres) were assessed at rest and during high-dose dobutamine (40 μg/[kg · min]). Expression of electron transport chain proteins was analyzed with isobaric tags for relative and absolute quantification.

Results: After revascularization, multidetector computed tomography confirmed severe left anterior descending stenosis and patent left internal thoracic artery graft. Regional function and blood flow normalized at rest; however, function in left anterior descending distribution remained depressed relative to remote regions, and myocardial blood flow in that region did not increase normally when challenged with high-work state. Concomitant with reduced maximal blood flow response in left anterior descending region was more than 40% reduction in electron transport chain proteins essential to adenosine triphosphate production.

Conclusions: Despite successful revascularization of hibernating myocardium, regional function and blood flow remained depressed during catecholamine stress. Electron transport chain proteins known to be downregulated during adaptive process within hibernating myocardium did not normalize after revascularization. These data demonstrate a potential bioenergetic cause of persistent dysfunction and heart failure within successfully revascularized hibernating myocardium. (J Thorac Cardiovasc Surg 2011;141:261-8)

Hibernating myocardium is a clinical entity that is manifested as viable but persistently dysfunctional myocardium in response to repetitive myocardial ischemia and is characterized by reduced regional blood flow without evidence of necrosis.[1,2] Experimental studies indicate that several adaptations occur in response to chronic hypoperfusion, including remodeling of resistance vessels,[3] increased glucose uptake,[4,5] altered expression of the calcium-regulatory proteins,[6,7] and apoptosis.[8] These responses to chronic ischemia may be a coordinated response to downregulate myocardial oxygen expenditure with the reduced oxygen availability and thus ensure myocyte survival. Clinically, revascularization of hibernating myocardium results in variable degrees of contractile recovery, and this heterogeneous response may reflect the inability of the myocardium to reverse these pleomorphic adaptations in full.

Since the seminal observation by Murray and colleagues[9] of ischemic myocardial preconditioning in the anesthetized canine model, mitochondria have been identified as being central to the understanding of myocardial protection.[10] The chronically ischemic mitochondria within the myocyte must adapt to hypoxia resulting from decreased blood flow, with a balanced reduction in energy and reactive oxygen species production to preserve myocardium.[11] As we have shown, adaptations to hibernation are multifactorial and involve alterations in the bioenergetics and proteomics of the mitochondria that allow the tissue to maintain viability but at reduced rate of energy production.[12] An inability to support electron flow through the electron transport chain (ETC) may lead to inefficient oxygen consumption during high work states, and a downregulation of ETC proton

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ET/BS

Abbreviations and Acronyms

ETC = electron transport chain
iTRAQ = isobaric tags for relative and absolute quantification
LAD = left anterior descending coronary artery
LITA = left internal thoracic artery
MDCT = multidetector computed tomography
MS = mass spectrometry
RCI = respiratory control index
TTE = transthoracic echocardiography

gradient could therefore result in a negative effect on maximal oxygen expenditure and subsequently on myocardial contraction. We believe that hibernating myocardium is a coordinated proteomic response to persistent ischemic stresses that result in preserved viability at the expense of maximal energy production.[11] The reversibility of these adaptations after revascularization has never been tested in suitable animal models. On the basis of the variable recoveries of hibernating myocardium in clinical studies, we postulated that mitochondrial proteomic adaptations, which limit oxidative injury and apoptosis at the expense of maximal oxygen consumption and functional recovery, may persist despite revascularization.

In a swine model of hibernating myocardium, we hypothesized that persistent myocardial wall motion abnormality in revascularized myocardium by off-pump coronary artery bypass graft (CABG) is associated with persistently depressed expression of key mitochondrial proteins involved with the ETC.

MATERIALS AND METHODS

Animal Model

All animal studies were approved by the institutional animal care and use committee of the Minneapolis VA Medical Center and conform to current National Institutes of Health (Guide for the Care and Use of Laboratory Animals, www.nap.edu/catalog/5140.html) and American Physiological Society guidelines for the use and care of laboratory animals.

Operative Technique

To create a model that reflects the clinical situation, we used a well-established swine model of chronic myocardial ischemia.[4,12] Two survival operations and a terminal operation were performed in 7 animals during a 16-week time course. The first operation created hibernating myocardium with the placement of a left anterior descending artery (LAD) occluder at time 0, the second operation surgically revascularized the myocardium with left internal thoracic artery (LITA) to LAD at 12 weeks, and finally the terminal procedure occurred 4 weeks after the bypass operation at 16 weeks.

Chronic hibernation model (8- to 10-kg pigs). Animals were sedated with tiletamine hydrochloride and zolazepam (Telazol, 4 mg/kg intramuscularly) and xylazine (2 mg/kg intramuscularly), intubated, and anesthetized with isoflurane (2%). Through a left thoracotomy approach, the chest and pericardium were opened in a sterile fashion. The LAD was dissected free, and a plastic c-shaped ring with an internal diameter of 2 mm encircled the LAD proximal to the first diagonal without occluding the vessel and was secured with sutures. The thoracotomy incision was closed in layers. The animal recovered for 12 weeks. We have previously shown that this vascular constrictor does create hibernating myocardium with a reduction in regional blood flow in the LAD region with time and loss of wall thickening in the anterior wall function 12 weeks after instrumentation.[4,12] To confirm hibernating myocardium, we documented proximal LAD stenosis by multidetector computed tomography (MDCT) and wall motion abnormality by transthoracic echocardiography (TTE) before the second operation. At 12 weeks, animals underwent the second survival operation, which was revascularization of the hibernating myocardium.

Revascularization model (50- to 60-kg pigs). Animals were again sedated, intubated, and anesthetized as noted previously. Coronary artery revascularization was performed through a midline sternotomy with the LITA dissected free from the chest wall. Lidocaine (1 mg/kg) was administered before opening the pericardium. The LAD distal to the site of c-ring occlusion was then exposed. The animal was heparinized (100 unit/kg). With an off-pump technique, the LAD was stabilized with a Titan Stabilizer (Estech, San Ramon, Calif). LAD perfusion was maintained with a Flo-thru intraluminal shunt (Synovis Surgical Innovations, St Paul, Minn). A LITA anastomosis to the LAD was performed just beyond the occluder with 8-0 Prolene suture (Ethicon, Inc, Somerville, NJ). On completion of the anastomosis, the shunt and LITA occluder were removed. Protamine was given to reverse the heparin, and the chest was closed in layers. A second MDCT and TTE were done before the terminal study at 16 weeks.

Terminal procedure (70- to 80-kg pigs). Four weeks after revascularization, a terminal study was performed. Animals were sedated, intubated, anesthetized, and monitored. Femoral and carotid arterial monitoring lines were placed to monitor blood pressure, obtain blood samples, and infuse intraventricular colored microspheres. Fluorescently labeled colored microspheres were injected into the left ventricle before and after a 5-minute infusion of dobutamine (40 μg/[kg $\cdot$ min]) to determine regional blood flows. A resternotomy was performed to excise the heart, and tissue samples were obtained for blood flow, proteomic, and histologic analyses.

Echocardiographic Analysis

Two-dimensional TTE measured regional myocardial function in the LAD and circumflex regions of the swine heart. The circumflex region (remote) was used as the control, with measurements made at standardized locations to allow comparisons between animals. TTE with and without dobutamine infusion was performed with a Vivid 7 Ultrasound machine (GE Healthcare, New York, NY) during deep sedation without intubation. Heart rate, heart rhythm, and oxygenation were monitored during the procedure. Wall thickening was measured at the right parasternal short-axis view in the posterior (circumflex) and anterior (LAD) walls. Wall thickening was computed as the difference between end-systolic and end-diastolic wall thicknesses and expressed as a percentage of end-diastolic thickness.

MDCT Analysis

MDCT was used to document LAD stenosis and confirm LITA graft patency. MDCT scanning of the hibernating and revascularized swine models was performed with the Brilliance 40 MDCT scanner (Phillips Medical Systems, Cleveland, Ohio) during deep sedation with Telazol without designated breath hold or β-blockade. Electrocardiographically gated image acquisition was used to reconstruct images retrospectively. A volume of 150 mL of contrast media was injected intravenously at a rate of 4 mL/s. Scanning was triggered automatically when contrast enhancement within the descending aorta reached a threshold level of 150 Hounsfield units. At least 10 phases were reconstructed for each study, and coronary analysis was done at either 75% phase or end-systolic phase. Image analysis was done with a dedicated workstation (Philips Extended Brilliance Workspace; Phillips Medical Systems).